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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 : G01N 1/30, 21/01, 31/22 — A1

(11) International Publication Number: **WO 86/ 00704**

(43) International Publication Date: 30 January 1986 (30.01.86)

(21) International Application Number: PCT/US85/01348

(22) International Filing Date: 17 July 1985 (17.07.85)

(31) Priority Application Number: 631,826

(32) Priority Date: 17 July 1984 (17.07.84)

(33) Priority Country: US

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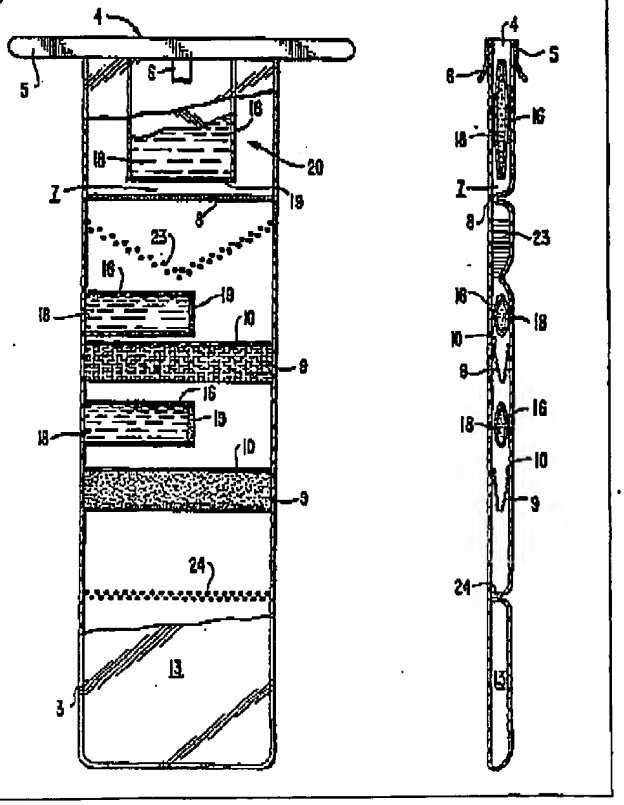
(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
*With international search report.*

(54) Title: METHOD AND APPARATUS FOR FILTERING PARTICULATE MATTER FROM FLUIDS OF BIOMEDICAL INTEREST AND EXAMINING SAME

(57) Abstract

A flexible, disposable pouch with internal filters (9), compartments (7, 13) and reagents (18), and having a transparent wall (1, 2) is used to filter, treat, and microscopically examine the filtrate or residue without reopening the pouch. The specimen fluid is moved from one compartment (7) to another (13), or through the filters (9), and the reagents (18) are released as a result of the pressure exerted as the pouch is rolled.



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# METHOD AND APPARATUS FOR FILTERING PARTICULATE MATTER FROM FLUIDS OF BIOMEDICAL INTEREST AND EXAMINING SAME

## BACKGROUND OF THE INVENTION

This invention finds application on the examination of fluids of biomedical interest for selected particulate matter. These applications, include but are not limited to, the examination of blood, urine, and fecal suspensions for human or microbial cells, or for parasitic organisms, and of air and drinking water samples for pollen and bacteria.

This invention relates in general to new and useful improvements in a flexible, disposable, plastic fluid collection pouch in which, in a simple manner, a specimen is passed through one or more filters and the residue and/or filtrate is analyzed. Filtration is aided and accelerated by rolling the pouch whereby fluid flow through the filters is maintained. Additionally, the residue or filtrate may be treated within the pouch by one or more selected materials contained separately within the pouch and the treated or untreated filtrate inspected, analyzed and/or microscopically viewed from within the pouch. Permanent and temporary seals of flexible plastic film form the chambers and containers of substances for treatment of the specimen filter residue and filtrate. A portion of the flexible plastic may be transparent in order to provide for visual control processing or analysis.

The treatment and analysis of a collected specimen poses a number of problems related to its exposure to the outside environment and to its transfer to different containers. There is retention of a portion of the specimen by the surfaces of the collection pouch as when a viscous liquid is poured from a collection chamber into a receptacle. The specimen is adulterated by substances within or adhering to the surfaces of a receptacle at the time of transfer. Volatile constituents of the specimen are lost with exposure to atmosphere and contents of atmosphere are absorbed by the specimen. Such considerations are of sufficient significance in some instances to require use of "clean rooms" with air cleaning systems and air locks.

The specimen may be noxious, lethal or contain irritant or infectious agents requiring special gloved handling in vented or laminar flow hoods. The use of the collection pouch of this invention as the treatment container and the container for viewing and analysis eliminates or greatly reduces many problems associated with transfer of the specimen between containers. Careful preparation of the collection chamber during its manufacture, so as to be free of dust or free of oxygen for example, eliminates the necessity of creation of special environments during speciment treatment and analysis in particular instance. Similarly, the need for specially ventilated space when specimen treatment requires use of noxious or dangerous agents, such as ammonia, or ether is lessened by use of the pouch of this invention. This is because these noxious agents would already be within a closed chamber. Even when specimen loss or adulteration and contamination of the environment is of little significance, the inclusion in the collection pouch of the means of filtration, treatment and analysis considerably decreases costs in comparison with the use of additional containers.

It is commonly desirable to examine a filtered specimen with the aid of a microscope. Generally speaking, the devices known in the art, the filtrate (either by itself, or on a portion of the filter) is removed from the device and transferred to a microscope slide. Like the transfer of the specimen for treatment from one container to another, the transfer of the specimen for analysis or examination increases the risk of contamination and loss.

Rapid particle filtration systems frequently require the use of separate devices and means, for example water pressure, positive or negative air pressure or centrifugation. For example, P.S. Visser and R.J. Pitchford (A Simple Apparatus for Rapid Recovery of Helminth Eggs from Excreta and Special Reference to Schistosoma mansoni, S.A. Medical Journal, 46: 1344:-1436, 1972) describe a method using water pressure to force matter through a series of filters fixed in a hard-sided cylinder so that a predetermined size of residue is obtained by opening the cylinder to remove the filter and filtrate.

This, of course, makes it more difficult or cumbersome to filter particulate matter in the field, since these auxiliary devices and means must be provided. The devices of this invention provide more rapid particle filtration than does gravity filtration without recourse to additional apparatus.

Membrane filtration per se and the use of multiple filters for sizing are known. Also, flexible plastic collection pouches have been described. The present invention, however, permits one to rapidly filter particles from a specimen in a flexible plastic pouch in a simple manner.

The device of the present invention is versatile in that it is easily adapted to a specific application by provision of appropriate filters and reactants. This versatility reduces the cost of customizing the device to a particular application. Treatment of particles on the surface of a filter for analysis is familiar, for example, C.A. Hunter and R. Burdorff, a Serologic Test for Typhoid Fever, Am. J. Clin. Path., 37: 162-167, 1962. However, the present invention allows treatment of filtered particles within the collection pouch thereby preventing their exposure to the outside environment.

Greenwald, US 3, 819, 045, Homgren, US 4, 318, 803, and Studer, US 3, 936, 373, describe flotation-type devices used, in conjunction with a filter, to separate parasitic ova from fecal matter. The device of the present invention does not rely on flotation, or on a plunger mechanism to impel the sample through the filter, though flotation prior to filtration may be accomodated within the device to increase yield of selected particles from semi-solid specimens. The cited references also are inferior to the present invention in that they do not integral means for microscopic viewing of the contents of the pouch.

Likewise, Warren (US 4, 162, 850) describes the filtration of eggs from urine onto a filter, the removal of the filter to a slide,

and the transfer of the slide to a microscope for viewing of the particles on the surface of the filter which is wetted to be made transparent. However, the present invention allows the microscopic viewing of filtered particles through the transparent filter and transparent walls of the flexible plastic collection pouch and thereby preventing its exposure to the outside environment.

Nuclepore Corporation markets a "Schisto-kit" incorporating the process of collecting and subsequently viewing particles on a filter membrane made transparent by wetting, which uses increased pressure on the specimen exerted manually by means of a syringe and attached filter-holder chamber. The kit is composed of several separate components and is disassembled to remove the filter and particles to a slide for microscopic viewing and which must be wetted to be viewed. Again, the device of this invention is not disassembled for microscopic viewing of the filtrate.

Both Studer, US 4, 025, 306, and Wilkins, US 3, 874, 851 describe methods of examining a blood sample for microfilaria. Neither teach use of a single unit in which the lysing and staining solutions are stored, the blood drawn in and mixed with the reagents, the product filtered, and the filtrate removed and examined microscopically. Other disadvantages of the Wilkins method are pointed out by Studer. Studer's approach requires a syringe, a disposable lysing staining solution vial, a disposable filter vial, and a microscope slide, all separate components. The sample must be transferred several times. Microscopic examination is performed outside the device.

Watanabe, US 4, 035, 304, describes a bag-type instrument for filtering the blood to remove denatured blood components and alien substances, utilizing filters having a U-, W-, or S- shape. While Watanabe recognizes that these configurations increase the effective filtering area of the filters, he does not recognize that the pressure distribution varies across these filters or that this feature can be significant in adapting the device to other applications. Additionally, Watanabe does not describe any means for

maintaining the flow of blood across the filter other than to suggest that the bag is incorporated in a blood transfusion device and that the flow may be restricted by a clamp. Finally, Watanabe is not concerned with either treatment of the blood components or with their microscopic examination.

Barta, US 4, 124, 449, describes a device for enumerating bacteria suspended in low numbers in an infusion containing particulate organic matter. While he provides for filtration and direct microscopic examination of the bacteria, his device is subject to a number of disadvantages. First, the bacteria are funneled into a well for counting. Because they are not confined to a plane, and because a microscope offers but a narrow depth of field, this renders direct microscopic examination more difficult than in the device of the present invention. Second, his device embraces several parts which must be machined and then fitted together. One of the parts is a slidable chamber. These aspects increase the cost of manufacturing and render the unit less robust. They also render it less suitable for use as a disposable device.

Stone, US 3, 539, 300, teaches use of a coarse prefilter in a body fluid collector and separator. Schlichting, US 3, 092, 221, describes a complex apparatus for monitoring atmospheric microbial population.

Johnson, US 3, 476, 515, describes an analytical test pack. He teaches use of a cumbersome technique for selectively rupturing reagent pods, in which some pods are first protected and all the remaining pods are then ruptured by squeezing the entire bag. In this invention, any reactant chamber may be caused to release its contents, without affecting the other reactant chambers, and without any added special protection for the latter which must be invoked by the user.

## SUMMARY OF THE INVENTION

The invention is a substantially flat, disposable, flexible, transparent plastic pouch, containing one or more filters preferably formed of two main sheets, for specimen processing, including, for example, collection, filtration, treatment and/or analysis (particularly for medical diagnosis) having chambers for collection, viewing, and containment of materials separated by permanent seals, or by frangible sealing means that rupture rapidly but smoothly.

One object of the invention is to provide a specimen processing pouch in which a specimen may be rapidly filtered by rolling the bag.

Another object of the invention is to provide a specimen processing pouch which prevents large particulate matter from interfering with filtration.

Another object of the invention is to provide a specimen processing pouch having chambers containing specimen treatment reagents which are unstable when mixed and are separated until the device is ready for use.

Another object of the invention is to provide a specimen processing pouch in which a sample is progressively driven through a series of filters, to differentiate particles of different sizes.

Another object of the invention is to provide a specimen processing pouch having chambers containing reagents separated from the specimen and one another for treatment of particles before or after they are isolated by filtration.

Another object of the invention is to provide a specimen processing pouch which is at least partially transparent to facilitate observation of the specimen, or to facilitate observation of the isolated particles, or to facilitate observation of the

reaction of analytical reagents with the specimen or isolated particles.

Another object of the invention is to provide a specimen processing pouch which accomodates prefiltration treatment of the specimen by centrifuge.

Another object of the invention is to provide a specimen processing pouch which accomodates prefiltration treatment of the specimen by flotation.

Another object of the invention is to provide a specimen processing pouch having a filtered sample collection chamber suitable for transport and storage to facilitate additional analysis.

Another object of the invention is to provide a specimen processing pouch for the isolation of particles from gases.

Another object of the invention is to provide a specimen processing pouch for isolation of particles which may be used without the need for laboratory facilities or for highly skilled technicians.

Another object of the invention is to provide a specimen processing pouch which may be used without transfer of material into or out of other containers.

Another object of the invention is to prevent contamination of the environment, other parties, or the operator.

It is the objective to provide an device and means of operation which is rapid, self-contained, inexpensive, accurate, and simple to operate.

## BRIEF DESCRIPTION OF THE DRAWINGS

Fig 1. The preferred embodiment for isolation of parasite ova from urine

1a. A frontal view
1b. A sagittal view
1c. A lateral view

Fig 2. A modification for urinalysis

2a. A frontal view
2b. A sagittal view

Fig 3. A modification for isolation of microfilaria from blood

3a. A frontal view
3b. A sagittal view

Fig 4. A modification for isolation of parasite ova from feces

4a. A frontal view
4b. A sagittal view

Fig 5. A modification for flotation and filtration of parasite ova from feces

5a. A frontal view
5b. A sagittal view

Fig 6. A modification for centrifugation and filtration of parasite ova from feces

6a. A frontal view
6b. A perspective view after lateral folding
6c. A perspective view after vertical folding
6d. The invention seated in centrifuge holder

Fig 7. The method of rolling the invention to maintain internal pressure

7a. The invention open to receive the specimen
7b. The rolling having broken the frangible seal and passing the specimen through the filters
7c. The filtrate having been forced through the inherent retaining filter into the waste compartment.
7d. The invention unrolled for positioning of the external frame for viewing

Fig 8. Variations of filter attachments

8a. A transverse or oblique attachment
8b. A J-shape attachment

## DESCRIPTION OF THE PREFERRED EMBODIMENTS

In this application, the term "filter residue" refers to the material retained by a filter, while the term "filtrate" refers to the material passed by a filter. In those embodiments of this invention in which several filters are employed in series, certain material may be a "filter residue" with respect to one filter and "filtrate" with respect of others.

The pouch of this invention is easily adapted to permit examination of either filtrate or filter residue, or both, depending on interest.

The term "reactant" is used in a broad sense to encompass not merely agents that actually react chemically with a substrate but any agent that alters a physical or chemical property of its substrate.

The device in its preferred embodiment is illustrated in Figure 1 and is a nearly rectangular, flexible pouch, preferably constructed of transparent plastic film approximately 0.004 inch in thickness. There is an upper leaf (1) and an under leaf (2). A perimeter seal (3) joins the leaves on the sides and bottom of the pouch leaving an aperture (4) at the top of the pouch. Attached to the external surface of the upper leaf adjacent to the aperture is a metal foil strip (5) at the midpoint of which is attached a small, flexible pull-tab (6). A similarly placed foil strip and pull-tab are attached to the under leaf. Additional metal foil strips (5) are attached horizontally to the upper and under leaves near the bottom of the pouch. The pouch is divided into three sections, the upper specimen compartment (7) separated from the central section by an horizontal frangible seal (8) which is a nonpermanent seal joining the upper and under leaves of the pouch. Below the central section is the filtrate compartment (13), terminating in a sealed point, the outlet (14).

In the central section of the pouch below the horizontal frangible seal is fixed a membrane filter (9) of a defined pore

size. The filter is folded so as to form a V-shape in cross-section, and its upper free margins are attached to the corresponding upper and lower leaves each by a horizontal filter seal (10). The lateral borders of the membrane filters are each sealed to their opposite halves as well as to the perimeter seal each by a lateral filter seal (11). An additional filter is similarly attached to the pouch below the first one and has a smaller pore size than the one above it. A filter reduction seal (12) begins at the perimeter seal on each side, just below the upper filter, and obliquely downwards and inwards to terminate just below the lower filter. This seal joins the upper and under leaves of the pouch, encompassing the filter.

By pulling apart the pull-tabs, the foil strips are bent outwards and the aperture is extended. The specimen to be tested is placed in the specimen compartment. The pouch then is sealed by rolling down the upper end of the pouch by rotating downwards the foil strip. The ends of the foil may be bent over to encompass the roll, holding it in place. Continued pressure exerted by further firm rolling of the pouch forces the specimen through all of the filter, leaving on the upper surface of each filter particles that are too large to pass through. The effective filtration area of the lower two filters is contained within the filter reduction seals. The filtrate passes into the waste compartment. If it is desired to remove the filtrate from the pouch, the outlet may be opened by cutting the plastic. After emptying, the outlet may be resealed simply by rolling upwards the lower end of the pouch on the lower foil strip. The particles retained on the filters may be viewed directly through the transparent pouch. The filters being transparent, allow direct light microscopy to be used. In viewing the particles across the V-shaped filter, the particles are concentrated since two layers of filter are being observed simultaneously.

A specific application of the concept of the device of the preferred embodiment is in the performance of urinalysis for parasite ova, such as in detection of schistosomiasis. Ten ml of urine is collected in the premeasured specimen compartment and the pouch

sealed by rolling the foil strips downwards. The continued rolling action ruptures the frangible seal and rapidly forces the urine through both filters down into the waste chamber. In this application, the upper filter is a 100 micron pore size polyester fiber weave while the lower filter is a 12 micron pore size filter membrane which is transparent when wet. The 30-70 micron in diameter schistosome ova may be microscopically viewed through the lower filter 1-2 minutes after sample collection.

A modification of the preferred embodiment to stain blood cells in urine is shown in Figure 2. Within the specimen compartment is a bladder (16), consisting of a small, flexible pouch attached to the upper part of the under leaf by a seal, the bladder attachment (17). Liquid contained within the bladder is the specimen diluting fluid (18). The bladder is closed across its lower margin by the bladder frangible seal (19). The bladder contains a stain that selectively stains polymorphonuclear leukocytes (white blood cells of the granulocyte series). This modification contains three filters, and a grid (15) is imprinted on the middle one. The grid consists of narrowly spaced horizontal and vertical lines.

In the specific application previously described, that of a device designed for urinalysis, 10 ml of urine is collected into the specimen compartment. The bladder contains stain in buffered solution. The frangible seal of the bladder then is ruptured by external pressure on the pouch in the area of the upper part of the bladder. This action allows the stain to mix with the specimen. External manipulation of the pouch assists the mixing process. Further rolling down of the pouch exerts pressure on the horizontal frangible seal of the pouch, exposing the first filter to the diluted specimen. Then, the urine is made to pass through the filters. Casts and white blood cells are retained by the upper 10 micron membrane filter, red blood cells by the middle 3 micron membrane, and bacteria form a residue on the lower 0.45 micron filter. The white blood cells particularly take up the stain, facilitating microscopic examination. Over the surface of the middle filter is imprinted a

grid. The grid allows for a semiquantitative estimate of size and numbers of particles.

A modification of the preferred embodiment for isolation and identification of protozoal and helminthic parasites in blood is illustrated in Figure 3. The perimeter seal (3) extends across the top encompassing a small nozzle as an aperture adaptation (21). One end is closed with a latex cap (22) and the other end is continuous with the combined bladder and specimen compartment (20).

The bladder (20) terminates in a bladder frangible seal (19). A single wedge-shaped filter (9) of 10 micron pore size is used. The lower diagonal edges of the membrane are fixed to the under leaf (1) by the filter reduction seals (12). The horizontal edge of the wedge is fixed to the upper leaf (2) by the horizontal filter seal (10).

A specific use of the modification for isolation of blood parasites is for detecting the presence of microfilaria. The latex cap (22) may be removed or a hypodermic needle used to penetrate it to inject 0.5 ml of blood through the nozzle aperture adaptation (21) and into the bladder (20). The bladder contains a blood cell lysing agent and differential stain. Optimal staining requires a 2-3 minute pause after which the bladder frangible seal (19) is opened by externally applied pressure and the blood passes through the filter leaving the microfilaria as a residue. The wedge filter concentrates the residue at the vertex of the wedge. The organisms may be microscopically identified.

A modification of the preferred embodiment for filtration of selected particles from stool samples is shown in Figure 4. A combined bladder and specimen compartment (20) with a bladder frangible seal (19) and containing a diluting fluid (18) has an aperture (4). A horizontal frangible seal (8) separates the specimen compartment (20) from the filtration system. The trough-shaped inherent prefilter (23) is formed of a series of permanent discontinuous seals extending from the perimeter seal of one side of

the pouch to that of the other. The inherent prefilter serves to eliminate debris larger than the spaces between the discontinuous seals (e.g., fibrous roughage). Below the inherent prefilter is a second bladder (16) with bladder frangible seal (19) containing a stain (e.g., iodine), and followed by a 100 micron pore size filter of woven polyester fibers. Below that is another bladder (16) with bladder frangible seal (19) containing a wash fluid, and followed by a 12 micron pore size membrane filter. The waste compartment (13) begins with the inherent retaining filter (24) which is formed of a series of permanent discontinuous seals spaced so as to allow fluid to pass when external pressure is applied but to prevent fluid passing due solely to hydrostatic pressure.

An example of its use is for isolation of Schistosoma mansoni ova from stool. Approximately 1 gm of fresh specimen is placed into the collection chamber and the opening sealed closed by tighly folding the foil strips. The bladder is broken, releasing 9 ml of 10% formalin and 1 ml of Triton X-100, so that the diluent can be mixed with the specimen. The formalin preserves the ova while the surfactant decreases adhesions of the specimen to promote mixing. The rolling action is resumed creating increased internal pressure in the collection chamber so that the frangible seal breaks to allow the mixture access to the inherent prefilter. The larger fecal debris will be retained by the inherent filter and the fluid portion forced through it as the rolling action continues. The second bladder contains an iodine stain. The resumed rolling action of the device again increases internal pressure, breaking the bladder frangible seal, and forcing the solution through the 100 micron woven dacron screening filter, which allows the 30-70 micron eggs to pass, and then forces the solution through the 12 micron pore size filter membrane, which retains the iodine stained schistosome eggs. The third bladder, containing a washing fluid, is broken as the rolling is continued, until all waste fluid passes through the bottom inherent retainer. The wash removes excess stain and clears the filter for viewing. The inherent retaining filter prevents waste fluid from passing back through the invention since external pressure must be applied to pass fluid through it.

A modification of the invention for flotation and subsequent filtration of selected particles from semisolid matter is illustrated in Figure 5. The specimen compartmnet (7) contains a flotation chamber (25) formed by a chamber perimeter seal (26) leaving a decanting port (17) at the top. the flotation chamber is divided by a horizontal frangible seal (8) to retain a flotation solution (28) at its bottom until time of use. A bladder (16) with a bladder frangible seal (19) is positioned beneath the flotation chamber and contains a washing solution. The filtration system consists of a trough- shaped inherent prefilter (23) and two filters with a bladder (16) between, the upper filter having a larger pore size than the lower one. Both filter membranes are attached transversely across the device and have no folds. Their lateral margins are attached at the perimeter seal and the horizontal filter seals to the upper leaf is at a different height from that to the under leaf. The filtration system is separated from the filtrate chamber by an inherent retaining filter (24).

A use of the modification for flotation prior to filtration is in isolation and identification of parasite eggs from stool specimen. A one gram specimen is placed into the flotation chamber of the specimen compartment. The foil top (5) then is closed and rolled down to close the decanting aperture (27). The horizontal frangible seal (8) of the flotation chamber is opened by applying pressure to it externally, and the flotation solution and specimen mixed by gentle massage of the pouch. A sugar solution with specific gravity of 1.2 to 1.3 floats Schistosoma mansoni ova while heavier matter settles to the bottom of the chamber. The top of the invention then is unrolled a few turns to open the decanting aperture (17) and the upper portion of the flotation mixture decanted. Downward rolling is resumed afterward and as the decanted fluid passes through the inherent prefilter (23), the bladder containing a washing solution (e.g., Saline) is burst, aiding in freeing particles from retained roughage. The larger particles are retained by the upper filter (80 micron polyester fiber weave) while smaller particles pass on to the lower (12 micron filter membrane) one. Schistosoma mansoni ova are

30-70 micron in diameter and are retained on the lower filter, and the iodine stain released from the last bladder (16) positioned between the two filters aids in their identification. The small size of the gaps in the inherent retaining filter require increased internal pressure for the filtrate to pass through it into the waste chamber. Afterwards, it prevents backwash of the fluid through the device. the residue on the lower membrane filter, including stained parasite ova, may be viewed microscopically for particle identification.

Figure 6a. shows an alternative modification for increasing the yield of parasite ova from a stool specimen by accomodating centrifugation. The filtration system is similar to that of Figure 4. The specimen compartment (7) and diluting fluid (18) contained by the horizontal frangible seal (8) is similar to Figure 5, as is the existence on one side of the specimen compartment (7) of a decanting port (27) between the perimeter seal (3) and the chamber perimeter seal (26). On the other side of the specimen compartment, a suspension fluid is contained by an extension of the chamber perimeter seal (26) and another horizontal frangible seal (8). Between the chamber perimeter seal (26) and the perimeter seal of this side is another decanting port (27).

A one gram specimen is intriduced through the opened aperture (4) and placed in the specimen compartment (7). The aperture then is closed and the top of the pouch locked by folding the foil strips (5) downward into a coil so that the coil encompasses the tops of the chamber perimeter seal (26) and bending the ends of the foil strips back over the coil. The diluting fluid then is released through the frangible seal by applied external pressure, and it is mixed with the specimen by gentle massage. Figure 6b. shows the top coiled and sides folded longitudinally. The longitudinal folding creates a columnar shape which is thicker at the top for containing the specimen and fluids. The bottom then is folded upward as shown in 6c. The folding is done back and forth across the width of the pouch and creating a stack of a series of downward facing panels. Each

filter is contained within a panel to equally distribute force on the filter when spun. The coiled and folded pouch fits within a centrifuge tube, as in Figure 6d., and all frangible seals intended remain intact under centrifugation are oriented so as to run substantially parallel to the force exerted during centrifugation.

After centrifugation the pouch is unfolded. The top then is uncoiled two turns, but the aperture need not be opened. Care should be taken in decanting the supernatant to distinguish between the two ports. The supernatant is decanted across the top of the horizontal frangible seal (8) containing the suspension fluid and into the decanting port (27) adjacent to it. This decanting port is continuous with the waste chamber and bypasses the filter system. The suspension fluid then is released through the horizontal frangible seal (8) and into the specimen compartment to resuspend the sediment. The resuspended sediment and suspension fluid is decanted through the other decanting port (27a). The pouch is rolled downward to control internal pressure as before. When most of the specimen has passed through the filters, the bladder frangible seal (19) is broken releasing an iodine stain to aid visualization of the schistosome ova trapped on the second filter (9).

Figure 7 illustrates the rolling of the invention and the position of a means (external frame) to facilitate microscopic viewing: 7a shows the invention open for receiving the specimen; 7b shows the device closed and rolled downward so that internal pressure forces the specimen through the inherent prefilter and filters; and 7c shows the invention rolled down to force the filtrate through the inherent retaining filter. In 7d, the invention has been unrolled a few turns in order to position an external frame over a filter for microscopic analysis.

The external frame positions the flexible bag on a microscope stage so that it can be accurately maneuvered and focused. The external frame consists of a pair of hinged frames being transparent or containing transparent windows. The pair of frames separated by

hinged means to accept and then encompass the width of the flexible bag when closed by means of a clamp. The rigid frame can be accommodated by any standard mechanical microscope stage. The frame also flattens the bag and filter membrane, and restricts the depth of field to allow adequate light transmission and ability to focus on individual cells through the transparent windows within the frame.

Figure 7 also illustrates the use of inherent filters as a means of controlling internal pressure and rate of fluid flow. The upper trough-shaped prefilter, consisting of wide gaps between discontinuous permanent seals, permits large increases in rate of flow with small increases in internal pressure. The lower retaining filter requires large increases in pressure to yield small increases in rate of fluid flow.

Modifications: The device of the preferred embodiment may vary in its dimensions and the material of which it is constructed. The device and components may be transparent in whole or in part. The configuration and position of compart- ments, chambers, filters, bladders, and seals may vary. This invention may be divided into one or more completely separate pouches, but containing mechanisms for communication between the separate pouches.

The illustrated preferred embodiment and modifications show vertical series of compartments and filters of decreasing pore size in vertical series. Said compartments and filters also may be in horizontal series to accommodate parallel processing of more than one specimen, or in horizontal series to accommodate different processes for a single specimen.

The aperture of this invention may be modified to accommodate different means of introducing or extracting materials through it. A wide aperture sealed with foil strips and a narrow aperture with a latex cap were illustrated. Different configurations, attachments and sealing means may be made to accommodate, for example, syringes, needles, pipettes, swabs and open mouth containers, or to collect a specimen from its source.

Inherent filters, consisting of gaps between discontinuous permanent seals of the leaves of the device may be constructed of different gap and seal lengths and may be positioned at various locations within the device. Inherent filters may compose the only filtration means of this invention. Inherent filters may serve device function in several ways. As was illustrated, gap size determines the size of particles retained when they are used as prefilters or filters and the smaller gap sizes retain fluid until a predetermined level of internal pressured is exceeded. The rate of fluid flow at different pressure levels may be controlled by the number of gaps composing the inherent filter. the level of pressure and rate of flow developed within different portions of the device may be predetermined by positioning inherent filters of specific gap sizes and number at different locations within the invention. An example of one such modification is the positioning of a wide gap inherent filter between a fiber filter and a more fragile filter membrane. Rate of fluid flow through the fiber filter may be accelerated by a high level of internal pressure which would burst the fragile filter membrane. The rate of fluid flow reaching the filter membrane is slightly retarded by the large gap inherent filter, but the level of internal pressure reaching the filter membrane is greatly reduced due to the spaced permanent seal closures of the inherent filter. Another example of the use of an inherent filter is for measuring a liquid specimen. Gap size and spacing may be predetermined to allow liquid to collect before being made to pass through it by application of external pressure or for a particular length of time (e.g., 30 seconds). Liquid volume then is measure by graduated markings on or in the specimen compartment or at other locations within the invention. Another use of inherent filters depends on their configuration. A trough-shaped and a horizontal configuration are illustrated. the trough-shape concentrates increasing amounts of internal pressure at its tip as the device is rolled doward which is useful for extracting fluid and particles from highly fibrous specimen. The horizontal configuration equalizes pressure across its extent. Other useful modifications of inherent filter configurations may be constructed, for example, semicircular,

zig-zag, or sawtooth wave forms distribute pressure and concentrate material in different useful patterns.

Filters are manufactured of many different materials. Most filter materials may be sealed directly to the flexible plastics composing the invention by means of heat, sonic or radio frequency welds, adhesives, etc. However, some filters seal with difficulty due to the nature of their material, thickness, or shape. Such filters may be fixed to panels to which they seal or conform the panels sealed in this invention. Filters may be positioned at various locations within this invention. One or more filter materials may be sealed into invention modifications containing no, one, or more inherent filters. The filters may be of various dimensions and pore size, and effective filtration area.

The preferred embodiment of this invention and its illustrated modifications show filters positioned in different orientations to fluid flow depending on the locations of their filter seals. The preferred embodiment illustrated V-shape and the illustrated modifications show wedge-shape and rectangular configurations oriented transversely to fluid flow. Different locations of horizontal, vertical and reductions seals may be used to produce a variety of different orientations. Examples of such modifications are shown in Figure 8. Figure 8a illustrates a transverse filter with no fold having the horizontal filter seal to the under leaf placed higher on the pouch than the horizontal seal to the upper leaf. In this modification, the lateral margins are encompassed within the perimeter seal. In Figure 8b, another modification is shown in which the filter is folded asymetrically, appearing J-shaped in cross-section. As in the above transverse configuration, the horizontal filter seals are at different positions relative to the pouch but are located so as to create a fold in the filter membrane. The lateral margins are encompassed within the perimeter seal to provide an extensive filtration surface and rate of flow. The lateral margins may be reduced by position- ing one or more filter reduction seals of the upper and lower leaves and encompassing

portions of a filter so as to concentrate residue on the encompassed portion.

Filters used in this invention may be opaque, translucent, transparent, or become transparent when wet. Grids constructed of lines at various distances from one another may be imprinted on one or more filters, on the under leaf or the upper leaf, or imprinted separately and used in or with this invention for aid in measurement and quantification of particles.

Sequestered within the pouch may be a variety of diluents, solvents, detergents, stains, fixatives, nutrients, and other reactants in gaseous, liquid and solid form. Sequestering may be by means of bladders of various sizes and shapes, con- structed of various materials, sealed into or simply placed within this invention. Sequestering may also be by construction of permanent and frangible seals of the leaves and components of the device itself.

Frangible seals may be used to sequester materials within the pouch or may be used to confine fluids during filtration or chemical treatments to portions of the pouch. An example of use in confinement of fluid was illustrated in the preferred embodiment, in which the specimen compartment was separated from the filtration system by a frangible seal. The specimen may be measured before filtration to a predetermined volume by filling the specimen compartment prior to breaking the frangible seal. Graduated markings on or in the specimen compartment may be used to aid measurement.

The filtrate or waste compartment outlet may be open, enclosed with permanent seals, or temporarily closed by other means until removal of collected material is desired. Compartments closed with permanent seals may be opened by cutting the plastic and resealed. Sealing means may be heat, sonic or other welds, or by glues and adhesive materials applied to the device. Sealing also may be by rolling as illustrated using foil strips, by clamps, or by apertures attached to the device to accommodate other means of filtrate

removal. Examples of other means of filtrate removal requiring aperture attachment are for the connection of a second filtration device of this invention or for a syringe, hyperdermic needle, or pipette.

The filtrate may be microscopically examined or otherwise visually analyzed. The filtrate may be chemically treated for analysis, as for example with dye indicators of pH or chemical strips contained within the filtrate chamber indicating levels of glucose.

Use of the invention of the preferred embodiment and modifications may be repeated if throughly washed and dried between uses. The washing may be done by passing cleansing fluids and rinses into the outlet and out of the aperture, and passing through the filters to clean residue from their surfaces and pores. Attachments may be fixed to the aperture and outlet to facilitate the flow of fluid during washing and rinsing.

While the rupturable compartments of the seals are adapted to be ruptured merely by manually rolling the pouch, further pressure may be exerted by use of conventional roller and winder means.

Variations of the invention allow isolation, identification and analysis of particles of selected sizes from liquid and semisolid specimens. Examples of uses are for biological materials and specifically for medical diagnosis. The preferred embodiment and its illustrated modifications are designed for isolation of Schistosome ova from urine or stool or microfilaria from blood samples. The illustrated designs of the invention also show capabilities for urinalysis in the search for other isolated particles such as blood cells and casts. Also, variations of the modifications allow isolation from intestinal specimens of parasites such as roundworms, hookworms and whipworms, and parts of worms such as proglottids of tapeworms, and helminthic larvae such as those of Strangyloides stercoralis. Variations of modifications also allow isolation of smaller organisms, for example protozoa such as coccidia and giardia,

and amebic trophozoites and cysts. Similarly, the invention as modified for isolation of microfilaria from blood could be used for dog heartworm or human filaria, or further modified for isolation of protozoa such as malaria and trypanosomes of African sleeping sickness and of Chagas' disease.

Claims

1. A disposable, flexible pouch for filtering particulate matter from fluids of biomedical interest and examining same which comprises:

(a) a specimen fluid compartment having closable aperture means whereby fluid may be introduced into the compartments;

(b) filtration means communicating with said specimen compartment; and

(c) a filtrate compartment which receives the filtrate passed by said filtration means.

Wherein the fluid may be caused to pass through said filtration means when the pouch is rolled from the specimen compartment end toward the distal end.

2. A disposable, flexible device for filtering particulate matter from fluids of biomedical interest and examining same comprising a pouch having an upper leaf and a lower leaf, sealing means for sealing together the upper and lower leaves; enclosed by said upper and lower leaves; closable aperture means communicating with the interior defined by said leaves, filtration means dividing said interior into a specimen compartment proximal to said aperture means and a filtrate compartment wherein the fluid may be caused to pass through said filtration means when the pouch is rolled from its proximal end.

3. The pouch of Claim 1, further comprising at least one internal means for mixing the specimen fluid with a substance prior to filtration and for a controlled time.

4. The pouch of Claim 1, further comprising at least one internal means for contacting particles retained by said filtration means with a substance at a controlled time.

5. The pouch of Claim 1, further comprising at least one internal means for mixing at least one of the filtrates yielded by said filtration means with a substance at a controlled time.

6. The pouch of Claim 1, further comprising a first reactant chamber and a second reactant chamber, respectively containing a first and a second reactant, said reactants reacting to yield a third reactant useful in the analysis of the specimen, said first and second reactants being more stable than said third reactant.

7. The pouch of Claim 1, further comprising a plurality of internal, independently releasable, compartmented reactant means.

8. The pouch of Claim 1, in which the filtrate compartment has a closable aperture means.

9. The pouch of Claim 1, in which the filtration means forms one side of the specimen compartment.

10. The pouch of Claim 1, in which the filtration means is separated from the specimen compartment by a frangible seal.

11. The device of Claim 1, in which said filtration means comprises at least one membrane filter.

12. The device of Claim 1, in which said filtration means comprises a plurality of filters arranged in series, with progressively finer pores.

13. The device of Claim 1, in which said filtration means includes a prefilter for retaining very large particles.

14. The device of Claim 1, in which said filtration means included filter reduction means for progressively restricting the lateral movement of the fluid.

15. The device of Claim 12, in which at least one filter has a substantially V-shaped in cross-section.

15. The pouch of Claim 1, in which a transparent portion of the pouch defines an observation window through which the filtrate or the residue may be observed.

17. A flexible, disposable pouch for the detection of parasitic ova in urine which comprises:

(a) a specimen compartment of predetermined size having closable aperture means;
(b) a filtration means comprising a first filter of pore size of about 100 microns, and a second filter of pore size of about 12 microns, said second filter being transparent when wet;
(c) said pouch being adapted so that the contents of said specimen compartment may be caused to flow into said filtration means by rolling said pouch; and,
(d) said pouch being adapted so that the particles retained on said second filter may be microscopically viewed without opening the pouch.

18. The pouch of Claim 1, adapted for the examination of blood cells in urine in which:

(a) the specimen compartment further comprises a subcompartment having a frangible seal, said subcompartment containing a diluent and a staining agent that selectively stains leukocytes; and,
(b) the filtration means comprises a first filter adapted to retain casts and white blood cells, and a second filter adapted to retain red blood cells, and a third filter adapted to retain bacteria.

19. The pouch of Claim 18, in which the first filter has a pore size of about 10 microns, the second filter has a pore size of about 3 microns, and the third filter has a pore size of about 0.45 microns.

20. The pouch of Claim 18, in which the retentive surface of the second filter bears grid means whereby estimating the size and number of particles retained thereon is facili- tated.

21. The pouch of Claim 1, in which the filtration means includes visualizable grid means for estimating the size and number of particles retained.

22. The pouch of Claim 1, adapted for the isolation and identification of microfilaria in blood, which further comprises lysing means and staining means.

23. The pouch of Claim 1, in which said specimen compartment further comprises a first subcompartment containing a lysing agent and a second subcompartment containing a staining agent, said subcompartments being rupturably sealed off from the remainder of said compartment.

24. The pouch of Claim 1, in which said specimen compartment further comprises a subcompartment containing both a lysing agent and a staining agent, said subcompartment being rupturably sealed off from the remainder of said compartment.

25. The pouch of Claim 1, in which the filtration means comprises a filter having a substantially V-shaped cross-section.

26. The pouch of Claim 1, in which said filtration means comprises a filter having an upper and a lower edge, the upper edge being sealed to the upper leaf of the pouch and the lower edge being sealed to the lower leaf of the pouch, said lower edge being displaced from said upper edge in the direction of flow.

27. A pouch for the filtration of selected particles from stool samples which comprises:

(a) a specimen compartment having closable aperture means and rupturable diluent means, and separated from the remaining

interior of the pouch by frangible sealing means;

(b) a prefilter means adapted to retain large fecal debris;

(c) a rupturable staining means;

(d) a first filter adapted to retain other fecal debris but to pass the selected particles;

(e) a rupturable excess stain removing means; and,

(f) a a second filter adapted to retain the selected particles, the pouch, and the second filter being so adapted that the retained particles may be visualized without opening the pouch.

28. The pouch of Claim 27, in which the prefilter is formed of a discontinuous permanent seal.

29. The pouch of Claim 27, in which the first filter has a pore size of about 100 microns and the second filter has a pore size of about 12 microns.

30. The pouch of Claim 27, further comprising a filtrate compartment adapted to receive the filtrate passing said first and second filter, and having said filtrate passing into said filtrate compartment through the gaps in a discontinuous seal, said gaps being so dimensioned and spaced so as to allow the filtrate to pass when external pressure is applied but preventing the filtrate from passing back due solely to internal pressure.

31. The pouch of Claim 1, in which the filtering means comprises an inherent filter.

32. The pouch of Claim 31, in which the inherent filter has a trough-shaped. semicircular, zig-zag, sawtooth, or other nonlinear configuration.

33. The pouch of Claim 1, in which the filtering means includes a filter having a J-shaped cross-section.

34. The pouch of Claim 1, wherein the specimen compartment is divided by a partition means into a flotation chamber and a prefiltration chamber, said flotation chamber being divided by a frangible seal into a proximal subchamber and a distal subchamber, said distal subchamber containing a flotation solution, and said proximal subchamber communicating with said aperture means, said partition means having closable means for decanting the supernate in said proximal subchamber into the prefiltration chamber, said prefiltration chamber communicating with said filtration means.

35. The pouch of Claim 34, in which said flotation solution has a predetermined specific gravity.

36. The pouch of Claim 34, in which said closable decanting means is a gap in said partition means which is closed or opened, respectively, by rolling or unrolling the pouch.

37. A centrifugable, flexible disposable pouch for the filtration and examination of particulate matter found in a fluid, and having at least one compartment having a frangible seal, wherein, said compartment is adapted to remain intact under centrifugation by orienting the seal so that it is substantially parallel to the force applied during centrifugation, said pouch being of such dimensions that it may be rolled or folded into a centrifuge holder.

38. A method of filtering and examinig particulate matter from a fluid which comprises introducing the fluid into a flexible pouch having a transverse filter, rolling the pouch to increase the fluid pressure and cause the fluid to flow through the filter, and examining the filtrate or residue thus obtained.

39. The method of Claim 38, in which the contents of the pouch are centrifuged prior to filtration.

40. The method of Claim 38, in which the contents of the pouch are internally treated prior to filtration.

41. The method of Claim 38, in which the residue or filtrate is internally treated prior to examination.

42. The method of Claim 38, in which the residue or filtrate is microscopically observed without opening the pouch.

43. The method of Claim 42, in which an external frame is fitted over the observation area of the pouch to facilitate microscopic observation.

44. The pouch of Claim 16, in which the observation window is adapted to permit microscopic observation.

45. The pouch of Claim 2, in which the aperture is defined by unsealed edges of the upper and lower leaf, and strip means are attached to said edges, whereby, the aperture may be closed.

46. The pouch of Claim 45, in which said strip means are adapted to serve as a key on which the pouch may be wound and secured from unrolling.

47. The pouch of Claim 1 further comprising winding means to facilitate rolling the pouch.

48. The method of Claim 38 in which roller means are used to facilitate rolling the pouch.

49. The method of Claim 38 in which the pouch is manually rolled.

50. The pouch of Claim 1 in which said reactant means are compartments containing a reactant and having a wall in common with the pouch, and adapted to release said reactant when external pressure is applied to said wall.

51. The pouch of Claim 1, further comprising specimen collection means communicating with said aperture means.

52. A method of isolating parasitic eggs from a stool specimen which comprises placing the specimen in a flexible pouch comprising a first and a second chamber, the first chamber having flotation means and the second chamber having filtration means, flotating the specimen in the first chamber, decanting the supernate into the second chamber, and filtering it in the second chamber to isolate said eggs in said filtration means.

53. A method of isolating parasitic eggs from a stool specimen which comprises placing the specimen in a flexible pouch, contrifugign the specimen with the pouch to leave a supernatant and a sediment, decanting the supernatant into a waste chamber within the pouch, resuspending the sediment, filtering the sediment within the pouch.

54. The pouch of Claim 26 further comprising progressively narrowing channel means directing the specimen fluid to said filter.

55. The pouch of Claim 54, adapted for the isolation and identification of microfilaria in blood, which further comprises lysing and staining means.

56. The pouch of Claim 29 in which the first filter is a woven filter.

57. The pouch of Claim 37, further comprising a specimen compartment, a supernatant outlet means, a rupturable compartment holding a suspension fluid, and a resuspended sediment outlet means.

58. A method of centrifuging and filtering a fluid for analysis which comprises:

(a) suspending the specimen if solid to obtain a fluid;
(b) placing the specimen in a flexible pouch comprising filter means, and sediment resuspension means;
(c) centrifuging the specimen;
(d) removing the supernate;

(e) resuspending the sediment by means of the internal sediment resuspension means, and

(f) filtering the resuspended sediment.

59. The pouch of Claim 57, further comprising a supernatant compartment communicating with said supernate outlet means.

60. The pouch of Claim 16, further comprising detachable flattening means.

61. The method of claim 38, further comprising the step of flattening the pouch by external frame means prior to examination.

FIG. IA.

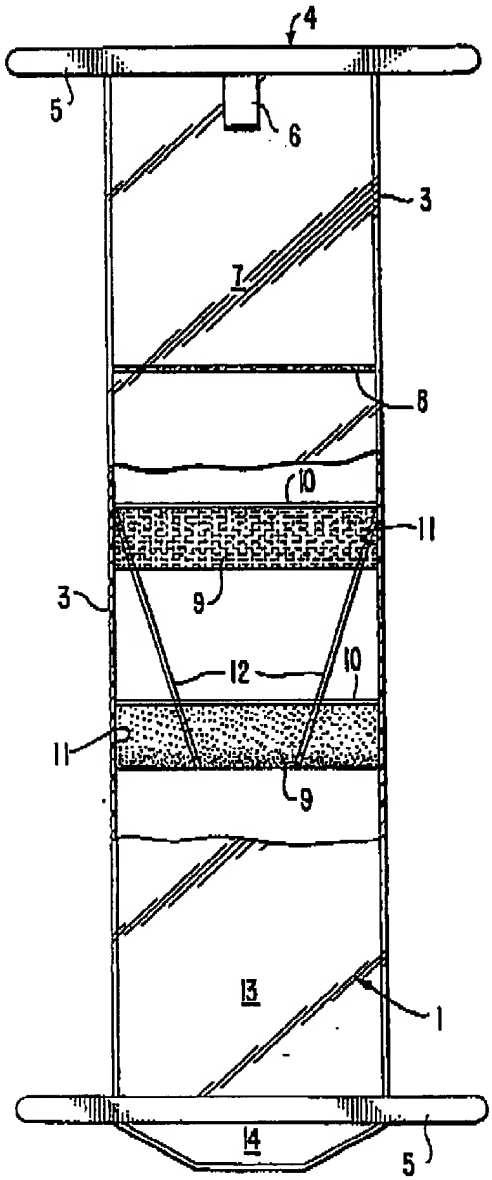


FIG. IB.

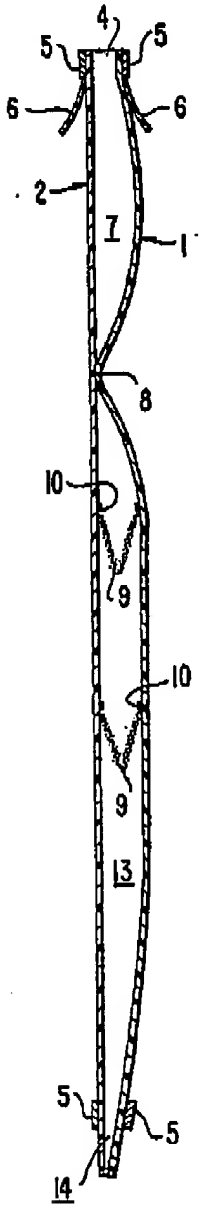


FIG. IC.

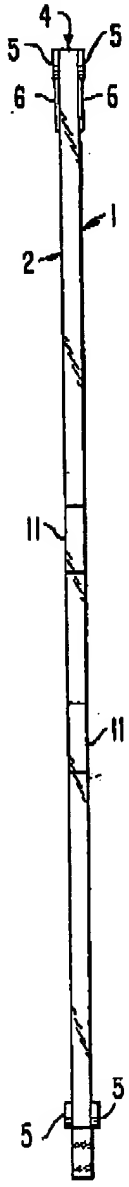

FIG. 2A.

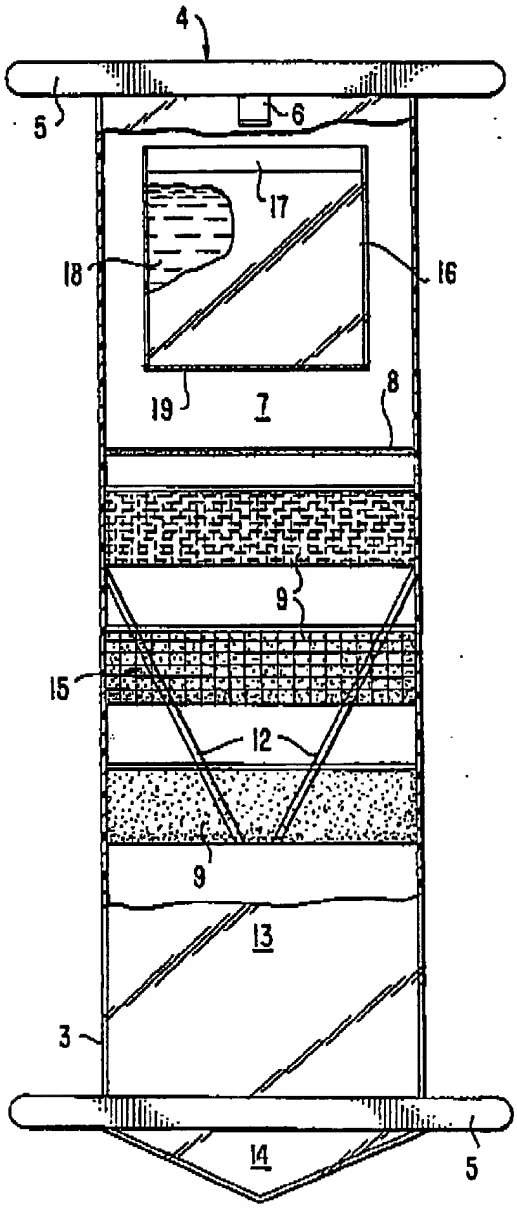


FIG. 2B.

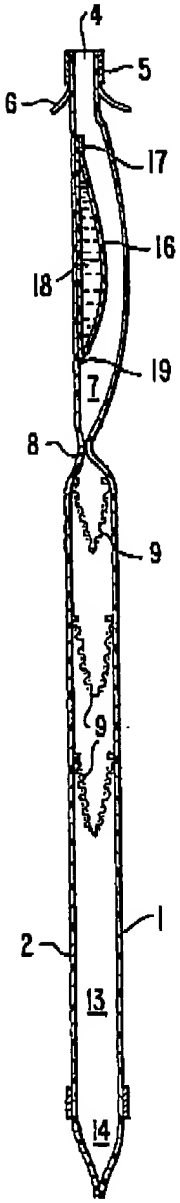

FIG. 3A.

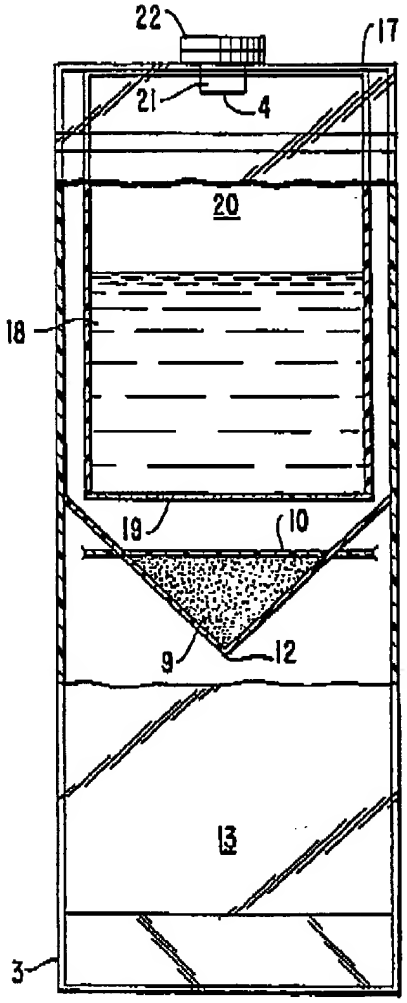


FIG. 3B.

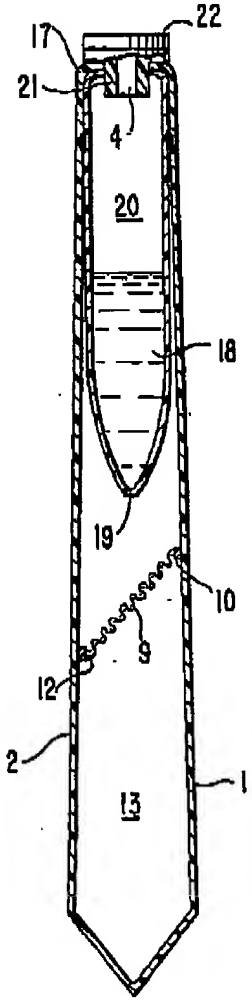


FIG. 8A.

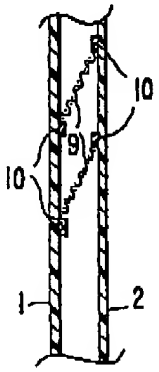


FIG. 8B.

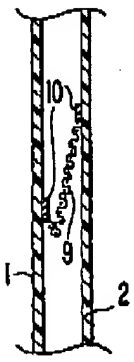

FIG. 4A.

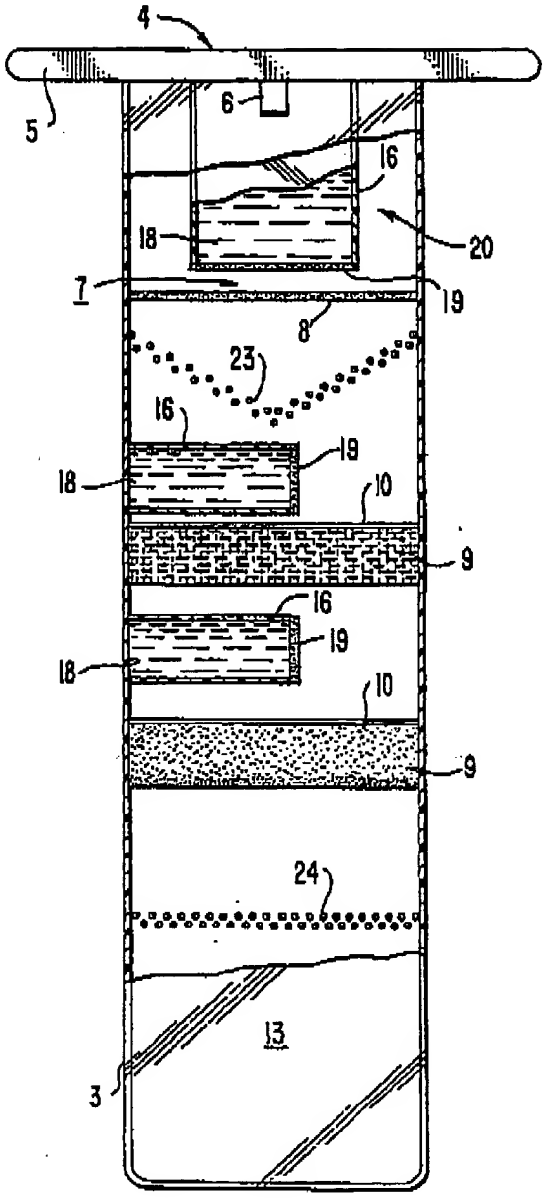
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FIG. 4B.

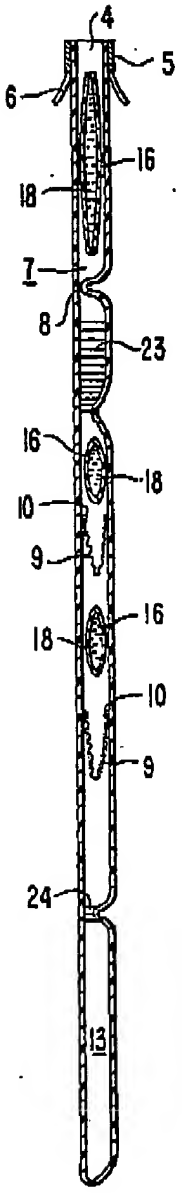
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FIG. 5A.

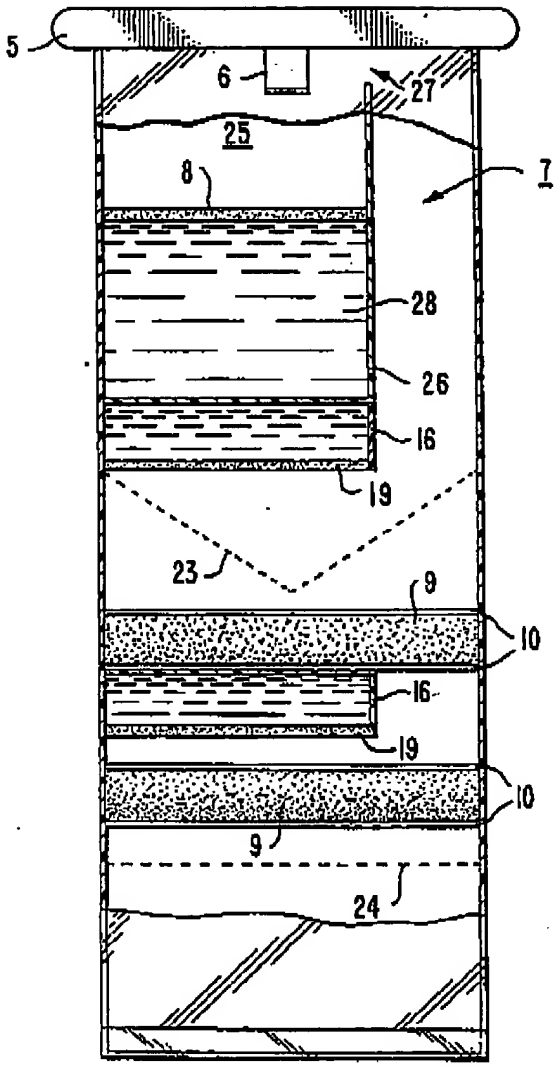
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FIG. 5B.

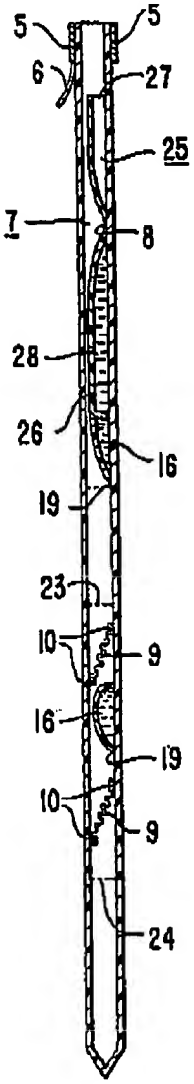
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FIG. 6A.

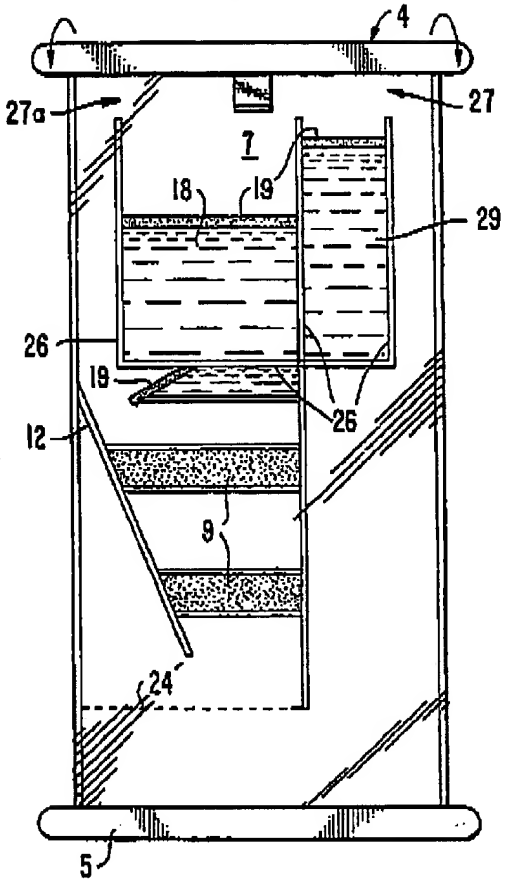



FIG. 6B.

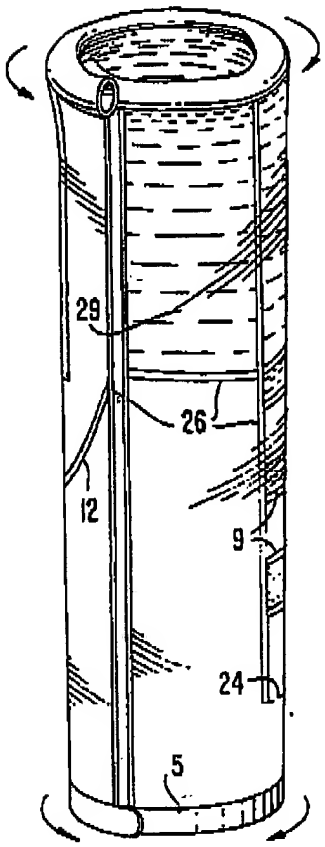



FIG. 6D.

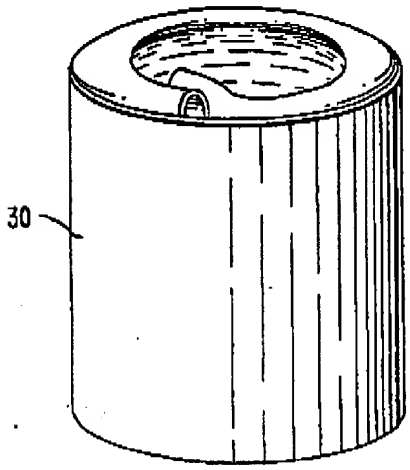



FIG. 6C.

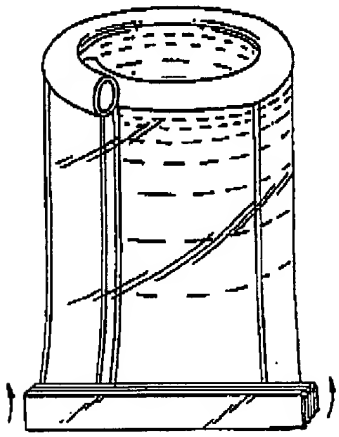


FIG. 7A.

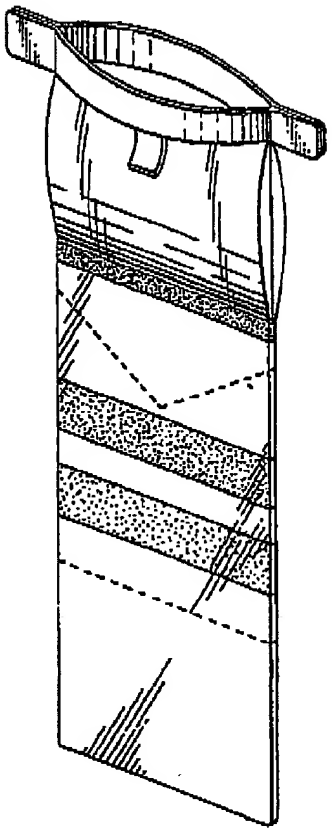


FIG. 7B.

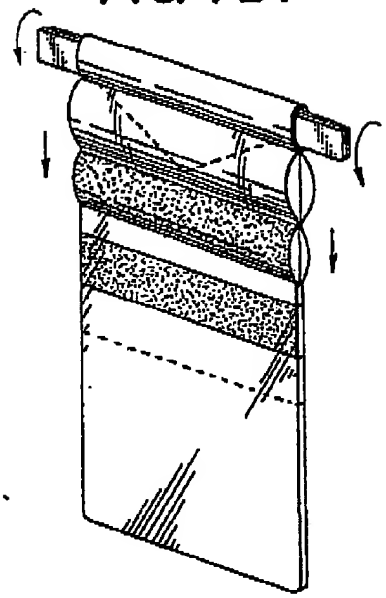


FIG. 7C.

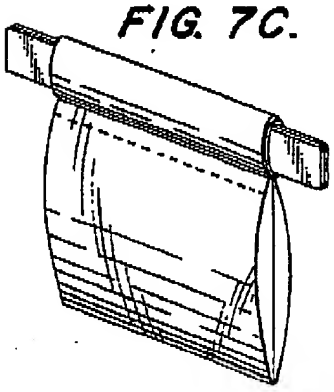
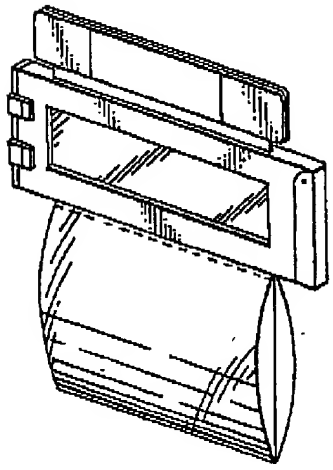


FIG. 7D.



# INTERNATIONAL SEARCH REPORT

International Application No PCT/US85/01348

## I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

U.S. CL. 422/58
INT. CL.[4] G01N 1/30, G01N 21/01, G01N 31/22

## II. FIELDS SEARCHED

Minimum Documentation Searched [4]

| Classification System | Classification Symbols |
|---|---|
| US | 422/61, 58, 72, 101, 102<br>436/45, 165, 177<br>210/94, 314, 317, 318, 323.2, 342, 446, 448, 497.01, 927<br>73/863.21, 864.91 |

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

## III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

| Category * | Citation of Document, [16] with indication, where appropriate, of the relevant passages [17] | Relevant to Claim No. [18] |
|---|---|---|
| A | US, A, 3,036,894, FORESTIERE, 29 MAY 1962 | |
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* Special categories of cited documents: [19]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

## IV. CERTIFICATION

| Date of the Actual Completion of the International Search [2] | Date of Mailing of this International Search Report [2] |
|---|---|
| SEPTEMBER 23, 1985 | 16 OCT 1985 |
| International Searching Authority [1] | Signature of Authorized Officer [20] |
| ISA/US | LYNN M. KUMMERT |

Form PCT/ISA/210 (second sheet) (October 1981)

International Application No. PCT/US85/01348

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _ ... ... because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ..... , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

1) Claims 1,3-16,18-36,44,47,50,51,54-56,60, drawn to an apparatus for filtering with chemical reactant means.
2) Claims 2,38-43,45,46,48,49,61, drawn to an apparatus and method for filtering.
3) Claim 17, drawn to an apparatus for filtering with microscopic viewing

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers: 1,3-16,18-36,44,47,50,51,54-56 and 60

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (supplemental sheet (2)) (October 1981)

International Application No. PCT/US85/01348

**FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET**

**V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]**

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ..... because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

**VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]**

This International Searching Authority found multiple inventions in this international application as follows:

4) Claims 37,57-59, drawn to filtering apparatus with centrifugation.
5) Claims 52-53, drawn to a method of solid/solid separation (stools/eggs) and filtering.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (supplemental sheet (2)) (October 1981)